IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
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Chin Ying HSIAO et al.	)	Group Art Unit: 1653
	)	
Application No.: 10/822,938	)	Examiner: S. M. Noakes
	)	
Filed: April 12, 2004	)	Confirmation No. 4416
	)	
For: NOVEL COLLAGEN PRODUCTION METHOD	)	

Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Seah June Nam, Ph.D., do hereby make the following declaration:

1. I am an inventor of the subject matter of this application and am employed as the Chief Scientific Officer with EcoDynamic BioLab, Inc.

2. I received my Masters of Science in 1994 from Yang Ming Medical University, Graduate School of Microbiology and Immunology. In 2002, I completed a doctorate in microbiology and immunology at the National University of Singapore, Temasek Life Sciences Laboratory. I have published several peer-reviewed journal articles in the area of microbiology. A copy of my curriculum vitae is attached as Exhibit A to this declaration.

3. My duties at EcoDynamic BioLab, Inc. include the development of methods to isolate collagen for therapeutic purposes. I am familiar with methods for the isolation of

collagen and have attempted to isolate collagen from various collagen-containing tissues. I am also familiar with the experiments disclosed in Examples 2-6 of the specification and Figures 2-6 (Exhibit B), which clearly detail the isolation of collagen monomers from various tissue sources using microbial fermentation.

4. I have read and am familiar with the pending claims and proposed amendments to these claims, as set forth in the Amendment and Response After Final, submitted herewith.

5. I have read and am familiar with the Office's rejection of claims 54-76 for lack of enablement, as set forth in the final Office Action dated November 4, 2005, and the Interview Summary of March 5, 2007.

6. I have read and am familiar with the arguments previously advanced by Applicants concerning the enablement of the claims. Specifically, I understand that the Office has asserted that it is unclear from the specification and claims how a skilled artisan isolates predominantly collagen monomers using the claimed invention. I further understand that while the specification includes several examples in which the isolation method of the invention is clearly described, the Office has suggested that there may be an essential missing element and step in the claimed methods that is not disclosed and that may account for the different amounts of the collagen forms shown in the figures.

7. Based upon my own experience and my study of the above, it is my belief and professional opinion that one of ordinary skill in the art could practice the claimed methods using the guidance provided in the specification. The specification discloses

five examples in which collagen monomers were isolated from various tissues using the methods of the invention. Further, while other forms of collagen may remain in the isolated collagen product, the examples clearly show that the disclosed methods produce the amount of collagen monomers required by the amended claims.

8. We, the inventors, describe five examples in the specification, which detail how the methods of the invention were used to isolate collagen monomers. (*See* specification, Examples 2-6, pages 18-25.) Examples 2, 3, and 4 describe the isolation of collagen monomers from avian, porcine, and shark tissues, respectively, using bacterial fermentation. Example 5 details the isolation of collagen monomers from avian tissues using yeast fermentation, while Example 6 details the isolation of type II collagen monomers from cartilage using bacterial fermentation.

9. Each of the five examples discloses experimental conditions in sufficient detail to enable one of skill in the art to practice the claimed invention. For instance, Example 2 details the amount and treatment of the avian collagen-containing starting material, culture conditions and preparation of the Gram (+) bacterium, and fermentation conditions, such as amount of loaded tissue, fermentation time, and rates of agitation and aeration. Subsequent to fermentation, Example 2 also clearly explains the conditions used to purify the collagen monomers using an acidic solution and enzyme preparation, filtration, delipidation, centrifugation, and precipitation. Finally, as in the other examples, Example 2 clearly discloses how the resulting collagen product is

analyzed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) for composition and purity.

10. The resulting collagen product in each of Examples 2-6 comprises collagen monomers weighing at least 10% of the weight of the total collagen in said collagen product, as the claims require. The composition of the collagen product produced in Examples 2-6 is evidenced by the SDS-PAGE gels in Figures 2-6, respectively. A copy of Figures 2-6 is attached as Exhibit B to this declaration.

11. The composition of the collagen product produced by the method of Example 3 is more clearly demonstrated by the attached figure labeled "For Fig. 3" (Exhibit C). I attest that the collagen product analyzed in that figure was produced by the experimental methods detailed in Example 3, but at a later date than the collagen product examined in the original Figure 3. The 8% SDS-PAGE gel used in the new figure was loaded with less sample volume than the 10% SDS-PAGE gel in original Figure 3, allowing for better separation and visualization of the collagen product. (*See* Exhibit C.)

12. The relative abundance of the β and γ collagen forms present in the collagen product differs between Examples 2-6. (*See* Exhibit B.) For convenience, labels for the β and γ forms of collagen have been added to the attached figures at their appropriate molecular weights, if previously missing in the original figures. These differences do not indicate that the specification and claims are missing an essential step. Such differences are to be expected because, for instance, the starting material (*e.g.*, avian

tissue in Example 1 and cartilage in Example 6), and microorganism (*e.g.*, yeast in Example 5 and bacterial fermentation in Example 2) vary between the examples. Most importantly, as indicated above, the α collagen form is present in the amount required by the claims and the isolation of the α form is clearly described in the specification and claims.

13. I believe that the specification and claims are not missing any essential step. Rather, the specification clearly describes to one of ordinary skill in the art how to make and use the invention. The examples and accompanying figures demonstrate that the collagen product produced by the isolation methods of the invention comprise collagen monomers in the amount required by the pending claims.

14. I hereby declare that the foregoing statements of fact set forth above are true, and that all opinions are believed to be true.

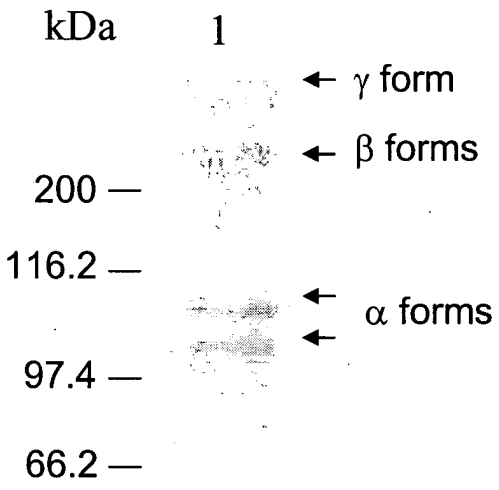
Dated: May 1st, 2007

By: _____
Dr. Seah June Nam

Attachments: Curriculum Vitae
Original Figures 2-6
New Figure "For Fig. 3"

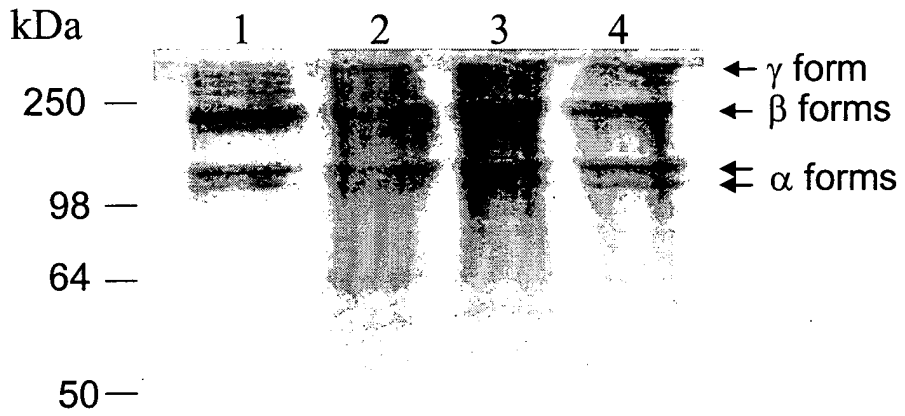
Curriculum Vitae

PERSONAL PARTICULARS	• Name: Seah June Nam • Address: 14F., No.14-1, Alley 20, Lane 142, Sec. 6, Roosevelt Rd., Wenshan District, Taipei City 116, Taiwan (R.O.C.) • Email: jnseah@collamatrix.com • Contact no: +886 2 7720 9988, +886 2 910197280
EDUCATION	• 1999-2002 Graduated with Ph. D. in Microbiology and Immunology, Temasek Life Sciences Laboratory, National University of Singapore • 1993-1994 Master of Science (MSc) Graduate School of Microbiology and Immunology, Yang Ming Medical University
EMPLOYMENT	• 1995-1996 Research assistant with Institute of Biomedical Science, Academia Sinica • 1997-1998 Assistant Research Officer with Animal Health Biotechnology Laboratory, Temasek Life Sciences Laboratories • 2003- Present Chief Scientific Officer with EcoDynamic BioLab
PUBLICATIONS	• Yu S, Ding H, Seah J, Wu K, Chang Y, Chang KS, Tam MF, Syu W. Characterization of a phage specific to hemorrhagic Escherichia coli O157:H7 and disclosure of variations in host outer membrane protein ompC. J Biomed Sci. 1998 Sep-Oct;5(5):370-82. • Seah JN, Yu. L, Kwang J. Localization of linear B-cell epitopes on infectious bronchitis virus nucleocapsid protein. Vet Microbiol. 2000 Jul 3;75(1):11-6. • Seah JN, Kwang J. Identification of H-specific determinants in flagellin of four Escherichia coli strains. Arch Microbiol. 2000 Jul-Aug;174(1-2):28-34. • Seah JN, Kwang J. Mapping of Escherichia coli H27-specific epitope from H-specific polypeptides. Clin Diagn Lab Immunol. 2001 Nov;8(6):1126-30. • Seah JN, Kwang J. Production of a monoclonal antibody specific to Escherichia coli H33 flagellin by using predetermined polypeptides. Arch Microbiol. 2002 Feb;177(2):192-6. • Seah JN, Kwang J. The N-terminal domain of RTX toxin ApxI of Actinobacillus pleuropneumoniae elicits protective immunity in mice. Infect Immun. 2002 Nov;70(11):6464-7. • Wang YT, Liu W, Seah JN, Lam CS, Xiang JH, Korzh V, Kwang J. White spot syndrome virus (WSSV) infects specific hemocytes of the shrimp Penaeus merguiensis. Dis Aquat Organ. 2002 Dec 10;52(3):249-59. • Seah JN, Kwang J. Localization of linear cytotoxic and pro-apoptotic epitopes in RTX toxin ApxIII of Actinobacillus pleuropneumoniae. Vaccine. 2004 Mar 29;22(11-12):1494-7.



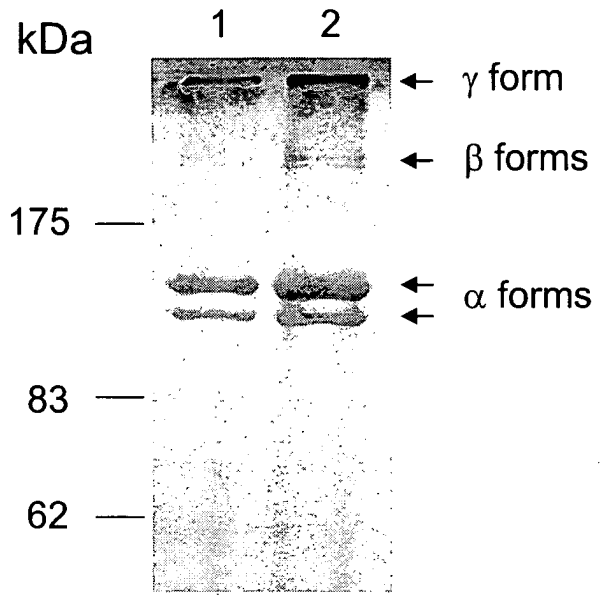
Electrophoresis of avian collagen on a 7.5% polyacrylamide gel. α and β denote different collagen configurations

FIG. 2



Separation of porcine collagen on a 10% polyacrylamide gel. α, β and γ denote different collagen configurations. Lanes 1, 2 porcine collagen, 20 μg and 40 μg; lanes 3, 4 commercial bovine collagen, 40 μg and 20 μg.

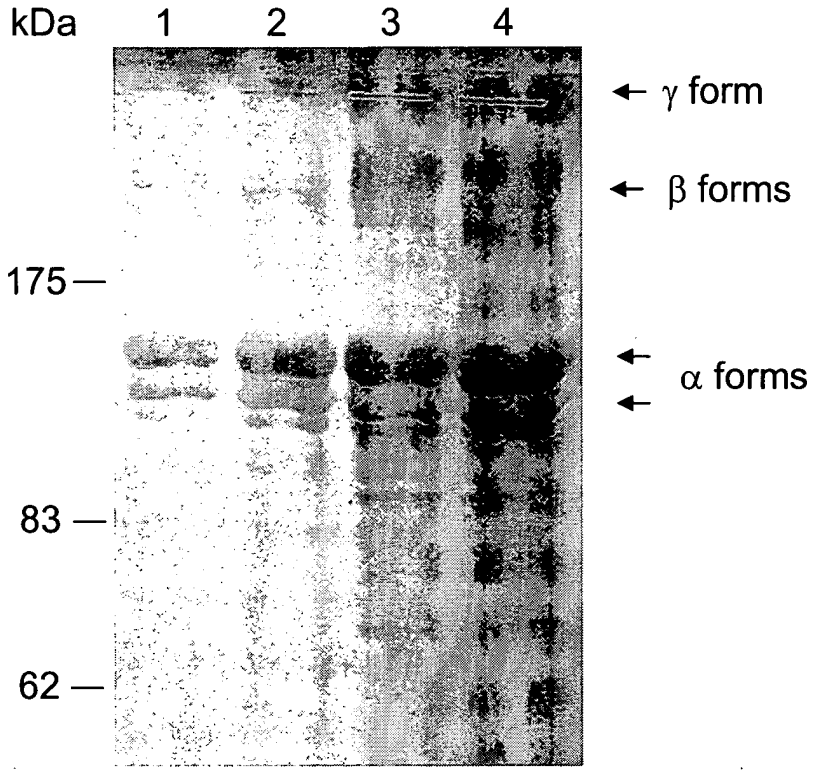
FIG. 3



8% SDS-PAGE

Lane 1: 10 μl
Lane 2: 20 μl

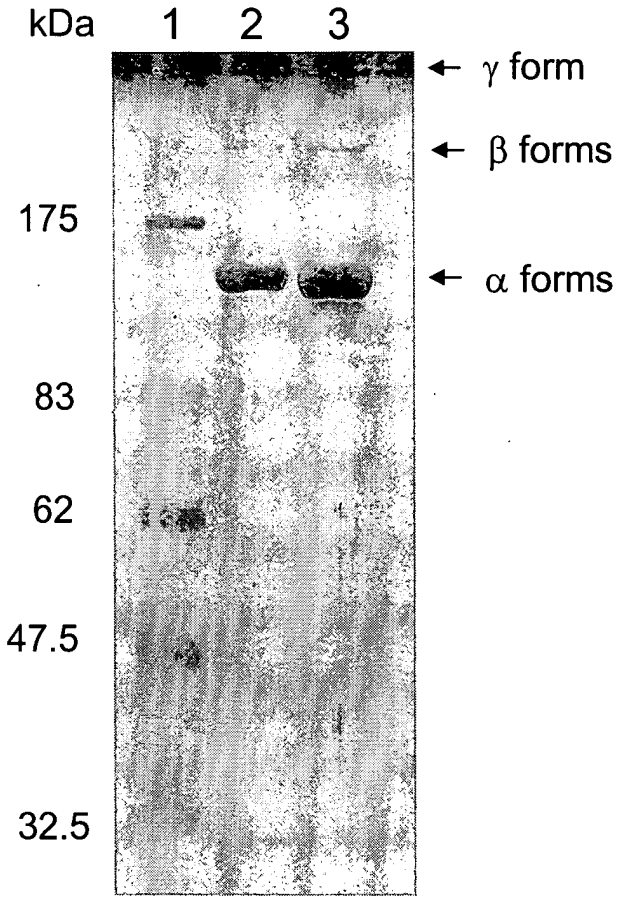
FIG. 4



8% SDS-PAGE

Lane 1: 5 μl
Lane 2: 10 μl
Lane 3: 20 μl
Lane 4: 40 μl

FIG. 5

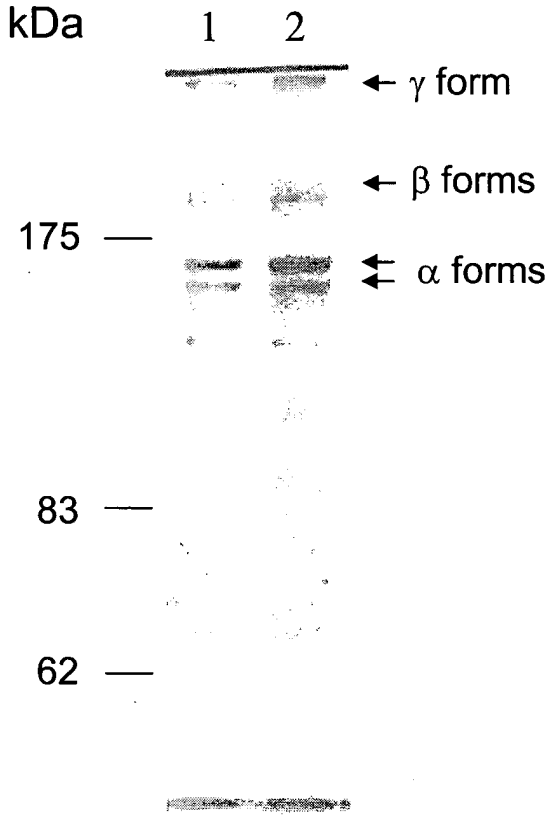


8% PAGE-SDS

Lane 1 : marker
Lane 2 : 5 μl
Lane 3 : 10 μl

FIG. 6

Separation of porcine collagen on a 8% polyacrylamide gel.



8% SDS-PAGE

Lane 1: 5 µl
Lane 2: 15 µl

For FIG. 3